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# Serum bile acids associate with liver volume in polycystic liver disease and decrease upon treatment with lanreotide

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## Abstract

**Background:** Polycystic liver disease (PLD) is a common extrarenal manifestation of autosomal dominant polycystic kidney disease (ADPKD). Bile acids may play a role in PLD pathogenesis. We performed a post-hoc exploratory analysis of bile acids in ADPKD patients, who had participated in a trial on the effect of a somatostatin analogue. Our hypothesis was that serum bile acid levels increase in PLD, and that lanreotide, which reduces liver growth, may also reduce bile acid levels. Furthermore, in PLD, urinary excretion of bile acids might contribute to renal disease.

**Methods:** With liquid chromatography-mass spectrometry, 11 bile acids in serum and 6 in urine were quantified in 105 PLD ADPKD patients and 52 age-, sex-, mutation- and eGFR-matched non-PLD ADPKD patients. Sampling was done at baseline and after 120 weeks of either lanreotide or standard care.

**Results:** Baseline serum levels of taurine- and glycine-conjugated bile acids were higher in patients with larger livers. In PLD patients, multiple bile acids decreased upon treatment with lanreotide but remained stable in untreated subjects. Changes over time did not correlate with changes in liver volume. Urine bile acid levels did not change and did not correlate with renal disease progression.

**Conclusion:** In ADPKD patients with PLD, baseline serum bile acids were associated with liver volume. Lanreotide reduced bile acid levels and has previously been shown to reduce liver volume. However, in this study, the decrease in bile acids was not associated with the change in liver volume.

## KEYWORDS

ADPKD, bile acid, biomarker, PLD

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## 1 | INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive bilateral renal cyst formation and a variable decline in kidney function.<sup>1</sup> An important extrarenal manifestation of ADPKD is polycystic liver disease (PLD) which is characterized by bile duct dilatation and the formation and growth of intrahepatic, fluid-filled biliary cysts (>20).<sup>2,3</sup> The overall prevalence of hepatic cysts in ADPKD is 83%; and 94% in those over 35 years of age.<sup>4</sup> Somatostatin analogues halt the progression of liver cyst growth by inhibiting cyclic adenosine monophosphate<sup>5-7</sup> and are part of the management in patients with symptomatic PLD. In a randomized clinical trial, the somatostatin analogue lanreotide reduced height-adjusted total liver volume (htTLV) by -5.91% in comparison with standard care.<sup>5</sup>

Bile acids (BAs), the end products of cholesterol metabolism, regulate numerous (patho) physiological processes in cholangiocytes.<sup>8,9</sup> In humans, the primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized and conjugated to either taurine or glycine in hepatocytes and secreted via bile into the intestines. Secondary BAs, the major ones in humans being deoxycholic acid (DCA) and lithocholic acid (LCA), are formed through modification of primary BAs by intestinal bacteria. More than 95% of the secreted BAs are reabsorbed in the intestine and recycled through the liver in the enterohepatic circulation. Only a very small proportion enters the systemic circulation. Circulating BAs are filtered by the glomerulus. Nearly all filtered BAs are subsequently reabsorbed in the proximal renal tubule.<sup>10-13</sup> Less than 1  $\mu\text{mol}$ /day is excreted with urine. When BAs accumulate in the liver, such as in cholestasis, they have cytotoxic effects and cause cholangiocyte cell damage.<sup>14</sup> Sulfation is an important mechanism for detoxification of BAs. It increases the water solubility, which contributes to faecal and urinary elimination.<sup>15,16</sup> Excess urinary elimination of BAs may contribute to tubular epithelial injury.<sup>17-21</sup>

The role of BAs in PLD has been explored to some extent.<sup>22,23</sup> In rats with a polycystic kidney and liver phenotype (PCK rats), BA levels are higher in the liver, in blood and in the kidneys than in wild-type controls.<sup>22</sup> Other studies, using the same animal model, also describe increased levels of intrahepatic,<sup>23,24</sup> serum<sup>23,24</sup> and urine BAs.<sup>23</sup> In PLD patients, data are limited to analysis of BA levels in cystic fluid, which were found to be higher than the levels detected in matched serum samples.<sup>22</sup> In humans, the role of BAs in progression of polycystic liver and/or kidney disease, regardless of a medical intervention, has not been studied.

Our aim was to examine whether BAs correlate with liver and renal disease progression. To this end, we

measured serum and urine BAs in a longitudinal, and well-characterized cohort of patients with ADPKD and PLD, who had participated in a controlled trial on the effect of lanreotide on disease progression. First, we studied the association between serum BAs and liver volume. Then we studied whether changes in serum BAs associate with changes in liver volume. In PLD patients treated with lanreotide, we compared serum BA levels before and after treatment. Lastly, we measured BAs in urine and explored whether changes over time correlate with a change in renal function. Our hypothesis is that serum BA levels increase in PLD, and that a somatostatin analogue, which reduces the rate of liver growth, may also reduce BA levels. We cannot assess causality in this study. Our other hypothesis was that urinary BA excretion may be higher in PLD and that this might contribute to the progression of renal disease.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and participants

In a post-hoc exploratory analysis in a subgroup of patients who participated in the DIPAK-1 study, we measured BA levels at baseline and after 120 weeks of follow-up. A description of the rationale, design and outcome of the DIPAK-1 study, 'a randomized controlled trial assessing the effect of lanreotide to halt disease progression in ADPKD' has been published previously.<sup>25</sup> The DIPAK-1 study included participants aged 18-60 years who had ADPKD and an estimated glomerular filtration rate (eGFR) of 30-60 mL/min/1.73m<sup>2</sup>. We restricted our analysis to patients with an eGFR  $\geq$ 45 mL/min/1.73m<sup>2</sup> at baseline to reduce the potential influence of decreased glomerular filtration rate on BA levels. We selected as cases (PLD group), 105 ADPKD patients with PLD and hepatomegaly, predefined in the DIPAK-1 trial protocol as having a total liver volume (TLV)  $\geq$ 2000 mL, i.e. not adjusted for height.<sup>5,25</sup> From the same study we selected as controls (non-PLD group), 52 ADPKD patients with a TLV <2000 mL. The controls were manually matched (1:2) for age, sex, eGFR and genetic PKD mutation (*PKD1* or *PKD2*). Patients were excluded if a serum sample was missing. A flow diagram of the inclusion of patients and the analyses is shown in [Figure S1](#).

The DIPAK-1 trial was centrally approved by the Medical Ethics Committee of the University Medical Center Groningen (reference: 2012/060), and additionally by the institutional review boards at each study site participating in the national DIPAK consortium (Leiden University Medical Center, Leiden; Radboud University Medical Center, Nijmegen; Erasmus Medical Center,

Rotterdam). The study was performed in adherence to the Declaration of Helsinki. All participants provided written informed consent.

## 2.2 | Study measurements and sample collection

Blood samples were stored at  $-80^{\circ}\text{C}$  until analysis. Serum creatinine ( $\mu\text{mol/L}$ ), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase ( $\gamma\text{GT}$ ) and bilirubin were measured using standard automated techniques. We used the following cut-offs for normal values of liver enzymes: serum AST  $<31\text{ mmol/L}$  (female (F)),  $<35\text{ mmol/L}$  (male (M)); serum ALT  $<34\text{ mmol/L}$  (F),  $<45\text{ mmol/L}$  (M); serum ALP  $<98\text{ U/L}$  (F),  $<115\text{ U/L}$  (M); serum  $\gamma\text{GT}$   $<38\text{ U/L}$  (F),  $<55\text{ U/L}$  (M); bilirubin total  $<21$  (both M and F) and bilirubin direct (conjugated)  $<5\mu\text{mol/L}$  (both M and F). Renal function was determined for each time point using the 2009 chronic kidney disease Epidemiology equation for eGFR.<sup>26</sup> The annual change in eGFR during follow-up was calculated as absolute progression rate in  $\text{mL/min}/1.73\text{m}^2$  per year using linear regression slopes through serial eGFR measurements. Standard methods were used to collect, process and store the over-night fasting, early morning void spot urine samples. Samples were collected in sterile containers and centrifuged directly at  $1000\text{g}$  for 10 min. The supernatant was processed into aliquots of 2 mL and stored at  $-80^{\circ}\text{C}$  until analysis. Sampling of fresh urine to measure pH was not part of the protocol. Urine bile acids ( $\mu\text{mol/L}$ ) were normalized to urine creatinine ( $\mu\text{mol/L}$ ) and expressed as the bile acid divided by creatinine ratio ( $\mu\text{mol/L}/\mu\text{mol/L}$ ). Furthermore, the fractional bile acid excretion was calculated using the following formula and expressed as a percentage:  $100 \times ([\text{urine bile acid in } \mu\text{mol/L} \times \text{plasma creatinine in ng/mL}] / [\text{plasma bile acid in ng/mL} \times \text{urine creatinine in } \mu\text{mol/L}])$ .

PKD mutation analysis was done by Sanger sequencing and multiplex ligation-dependent probe amplification.<sup>27</sup> MRI data were obtained at baseline, week 120 (end of treatment) and week 132 (end of study) to assess TKV and TLV using a standardized abdominal MRI protocol without the administration of intravenous contrast. TKV and TLV were assessed by classical volumetry and adjusted for height by dividing TKV or TLV by patient height in metres (htTKV and htTLV).<sup>25</sup> Patients' liver disease severity was classified based on htTLV as mild ( $<1600\text{ mL/m}$ ), moderate ( $1600\text{--}3200\text{ mL/m}$ ) or severe ( $>3200\text{ mL/m}$ ).<sup>5,28</sup> To evaluate liver volume progression, the change in htTLV between baseline (prior to treatment) and week 120 was calculated and expressed in absolute change and percent change.

## 2.3 | BA measurements

*Serum:* We measured a set of eleven BAs in serum with the following classification; primary unconjugated BAs: cholic acid (CA) and chenodeoxycholic acid (CDCA); primary conjugated BAs: glycine-conjugated cholic acid (GCA), glycine-conjugated chenodeoxycholic acid (GCDCA), taurine-conjugated cholic acid (TCA) and taurine-conjugated chenodeoxycholic acid (TCDCA); secondary unconjugated BAs: ursodeoxycholic acid (UDCA), deoxycholic acid (DCA) and hyodeoxycholic acid (HDCA); secondary conjugated BAs: glycine-conjugated deoxycholic acid (GDCA) and taurine-conjugated deoxycholic acid (TDCA).<sup>29</sup>

Bile acids were analysed as described elsewhere.<sup>30</sup> Briefly,  $10\mu\text{L}$  of an internal standard solution ( $500\text{ ng/mL}$  and  $290\mu\text{L}$  methanol) was added to  $100\mu\text{L}$  of serum. Samples were vortexed (5s) and stored at  $-20^{\circ}\text{C}$  for 20 min. Samples were centrifuged (10 min;  $18,213\text{g}$ ;  $4^{\circ}\text{C}$ ). Then supernatant was dried under a gentle stream of nitrogen and reconstituted by addition of  $40\mu\text{L}$  methanol followed by vortexing (5s) and sonication. Sixty  $\mu\text{L}$  of water was added, and samples were vortexed (5s) and centrifuged (10 min;  $18,213\text{g}$ ;  $4^{\circ}\text{C}$ ). About  $80\mu\text{L}$  supernatant was transferred to glass micro vial inserts, which were placed in the autosampler. For this project, pooled samples were included in every batch to ensure the data quality when comparing batches. These pooled samples were made by taking  $100\mu\text{L}$  plasma from each sample in the first randomized batch and pooling. The sample was vortexed (5s), and  $100\mu\text{L}$  aliquots were made. These pooled samples were extracted in the same way as the project samples. BAs were analysed using liquid chromatography-mass spectrometry (LC-MS/MS) analysis on a Shimadzu Nexera series system, consisting of two LC-30AD pumps, a SIL-30AC autosampler and a CTO-20AC column oven, coupled to a Sciex QTRAP 6500 with an electrospray ionization (ESI) source. All measurements were performed using an Acquity UPLC BEH C18 column,  $1.7\mu\text{m}$ ,  $2.1\text{ mm} \times 100\text{ mm}$  with a VanGuard pre-column (Waters). Mobile phase A consisted of 90% water with 10% methanol, containing 10 mM ammonium acetate adjusted to pH 8.5 using ammonium hydroxide. Mobile phase B consisted of methanol with 10 mM ammonium acetate with addition of  $120\mu\text{L}$  ammonium hydroxide. The flow rate was set to  $.5\text{ mL/min}$ . The chromatographic gradient started at 20% B and was held constant for .1 min, after which it was linearly increased to 55% B at .28 min, linearly increased to 73% B at 6.28 min, linearly increased to 100% B at 8.08 min and held constant for 2 min. The injection volume was  $5\mu\text{L}$ , and the oven temperature was kept at  $45^{\circ}\text{C}$ . For the detection of bile acids, the system was operated in the negative ESI mode. The following source settings were used: ion spray voltage,  $-4500\text{ V}$ ; temperature,  $600^{\circ}\text{C}$ ; curtain gas, 30;

source gas 1, 30; source gas 2, 60; collision gas, medium; entrance potential,  $-10$ . For each BA and IS, specific MRM transitions were monitored.

**Urine:** We measured a set of six primary, (un)conjugated BAs in urine: CA, CDCA, GCA, GCDCA, TCA and TCDCA. BAs were essentially analysed as above and previously described<sup>31</sup> using UPLC-hybrid Quadrupole-Orbitrap Mass spectrometry in ESI-negative mode on a configuration consisting of an Acquity UPLC (Waters) coupled with a Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific). In short, urine samples were manually ordered in random order (study number and time point), thawed and transferred into 96 deep-well plates. They were cleansed by centrifugation and then spiked with glycochenodeoxycholic-2,2,4,4-d<sub>4</sub> acid as internal standard. Separation was achieved by reversed phase chromatography using an Acquity UPLC BEH C18 1,7  $\mu\text{m}$ : 2.1  $\times$  50 mm column and a buffer system consisting of ammonium acetate, acetonitrile and stearic acid.

## 2.4 | Statistical analysis

Continuous variables with normal distribution were expressed as mean  $\pm$  standard deviation (SD). Non-normally distributed variables were summarized by median and interquartile range (IQR). Categorical data were given as proportions. Differences between baseline characteristics were analysed using an independent *t* test in case of normally distributed data, a Mann–Whitney *U* test when data were not normally distributed and a chi-square test in case of categorical data. BA data were log-transformed to meet the assumption of normality. The Shapiro–Wilk test was used to assess the efficiency of the transformation.

Cross-sectional analyses were performed on baseline serum and urine samples. **Serum analyses:** A Pearson correlation coefficient was computed to assess the relationship between BAs and htTLV in the total study population. Liver volume was also studied as a categorical variable in PLD patients. The differences in BA levels between mild and severe disease were analysed with the Mann–Whitney *U* test. An independent *t* test was used to analyse differences in BAs between PLD and non-PLD patients. **Urine analyses:** Similar statistical tests were used to assess the correlation between BAs and baseline eGFR, htTKV or annualized eGFR slope and to study differences in BAs between PLD and non-PLD patients. The different analyses are summarized in [Figure S1](#).

Longitudinal analyses were performed on baseline serum and urine samples. **Serum analyses:** To determine differences in BAs between a baseline sample and a subsequent sample after 120 weeks in PLD and non-PLD patients,

we used a paired-sample *t* test. Analyses on liver growth and the response to an intervention were only performed in PLD patients stratified by treatment group. Changes in liver volume between both time-points in patients who received lanreotide or standard care were studied using the Wilcoxon test and changes in BAs using paired-sample *t* test. A Pearson correlation coefficient was computed to assess the relationship between baseline BAs and change in htTLV and between changes in BAs and a change in htTLV. **Urine analyses:** Similar statistical tests were used to study differences in BA levels between the two time-points; and to assess the correlation between follow-up BA levels and baseline eGFR, htTKV or annualized eGFR slope in both treatment groups.

For all analyses, two-sided *p* values  $< .05$  were considered to indicate statistical significance. Data analysis and visualization were performed with R versions 4.2.3 and 4.3.0. and Python version 3.10.6. The following packages were used for the data analysis and visualization: *tidyverse* (2.0.0.), *patchwork* (1.1.2.), *haven* (2.5.2.), *broom* (1.0.5.) and *ggplot* (3.4.2.).

## 3 | RESULTS

### 3.1 | Study population

We included 105 patients with PLD (61% female, mean age  $48 \pm 7$  years, mean eGFR  $57 \pm 8$  mL/min/1.73m<sup>2</sup>) and 52 sex-, age-, eGFR- and genetic mutation-matched non-PLD patients. Baseline characteristics are reported in [Table 1](#). Median htTLV was 1487 mL/m (IQR 1252–1921) in PLD and 969 mL/m (IQR 896–1043) in non-PLD patients. In the PLD group, serum levels of  $\gamma$ GT were higher (mean  $57.0 \pm 43.0$  U/L,  $p < .001$ ), and 47% of these patients showed  $\gamma$ GT levels above the upper limit of normal, while this was only 10% in the non-PLD group. Bilirubin levels were similar in both groups and remained within normal ranges in 96% of PLD patients. Liver volume was neither correlated with kidney volume in the total study population ( $r = -.085$ ,  $p = .3$ ), nor in the PLD patients ( $r = -.158$ ,  $p = .1$ ).

### 3.2 | Serum BAs

3.2.1 | Analysis of the correlation between baseline BAs and htTLV ( $n = 157$ ) and comparison of baseline BAs in PLD ( $n = 105$ ) and non-PLD ( $n = 52$ ) patients

Descriptive data of all BAs are summarized in [Table S1](#). In an analysis of all ADPKD patients, serum levels of the

**TABLE 1** Baseline characteristics of patients with and without PLD in the context of ADPKD ( $n = 157$ ).

Variable	PLD patients ( $n = 105$ )	Non-PLD patients ( $n = 52$ )	$p$ Value <sup>a</sup>
htTLV, mL/m	1487 (1252–1921)	969 (896–1043)	<.001
Female sex, $n$ (%)	64 (61)	32 (62)	.5
Age, years	48 ± 7	47 ± 7	.9
Height, cm	176 ± 10	174 ± 9	.1
Weight, kg	85 ± 19	79 ± 14	.1
BMI, kg/m <sup>2</sup>	27 ± 5	26 ± 5	.3
SBP, mmHg	133 ± 14	132 ± 12	.9
DBP, mmHg	82 ± 9	81 ± 9	.4
Use of a statin, $n$ (%)	15 (14)	3 (6)	.1
AST, mmol/L	24.7 ± 6.6	22.5 ± 4.6	.02
ALT, mmol/L	26.8 ± 15.3	19.9 ± 7.4	.002
ALP, U/L	71.2 ± 22.5	64.1 ± 19.9	.1
γGT, U/L	57.0 ± 43.0	27.4 ± 20.2	<.001
Bilirubin direct, μmol/L	3.2 ± 1.6	3.1 ± 1.0	.7
Bilirubin total, μmol/L	9.6 ± 3.8	9.4 ± 3.6	.8
Total cholesterol, mmol/L	4.9 ± 1.0	5.2 ± 1.0	.05
htTKV, mL/m	1228 (658–1457)	1065 (653–1553)	.9
eGFR, mL/min/1.73m <sup>2</sup>	57 ± 8	58 ± 7	.7
eGFR slope, mL/min/1.73m <sup>2</sup> per year	−3.2 (−4.6 to −1.5)	−3.7 (−5.2 to −1.2)	.4
Urine ACR, mg/mmol	4.0 (1.6–9.4)	3.3 (1.3–7.0)	.1
PKD mutation, $n$ (%)			.6
PKD1	87 (83)	43 (83)	
PKD2	18 (17)	9 (17)	
Intervention group, $n$ (%)			.3
Lanreotide	54 (51)	23 (44)	
Standard care	51 (49)	29 (56)	

Note: Values in mean ± SD or median (interquartile ranges) in case of non-normal distribution.

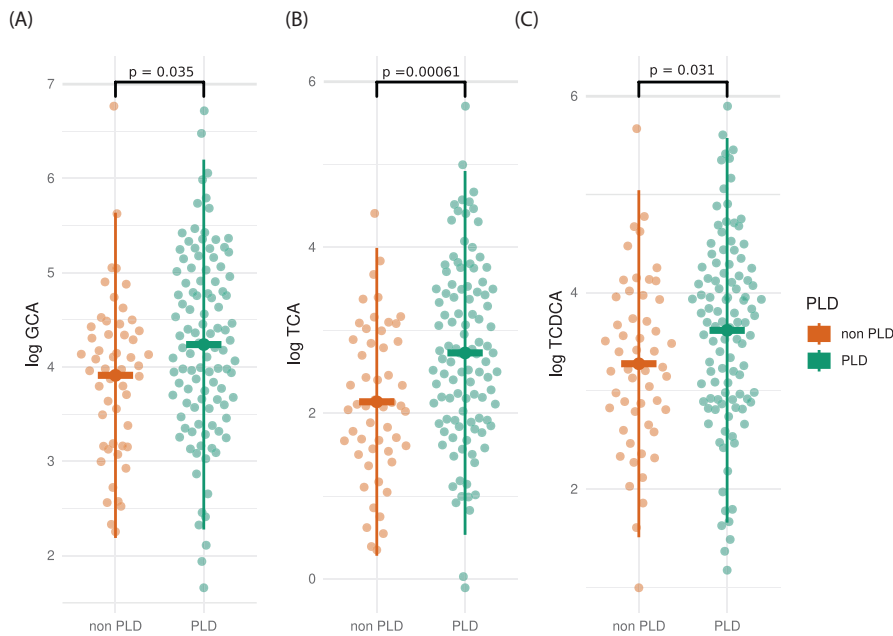
Abbreviations: ACR, albumin-to-creatinine ratio; ADPKD, autosomal dominant polycystic kidney disease; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; htTKV, total kidney volume adjusted for height; htTLV, total liver volume adjusted for height; PLD, polycystic liver disease; SBP, systolic blood pressure; γGT, gamma-glutamyltransferase.

<sup>a</sup> $p$  Values were calculated using the independent  $t$  test or Mann–Whitney  $U$  test.

primary conjugated BAs TCA ( $r = .390$ ,  $p < .001$ ), TCDCA ( $r = .346$ ,  $p < .001$ ), GCA ( $r = .222$ ,  $p = .004$ ) and GCDCA ( $r = .230$ ,  $p = .003$ ) were positively correlated with baseline htTLV (Figure S2). PLD patients had significantly higher levels of serum GCA (1.2-fold,  $p = .04$ ), TCA (1.9-fold,  $p < .001$ ) and TCDCA (1.6-fold,  $p = .03$ ) than non-PLD patients (Figure 1). Levels of secondary (un)conjugated BAs did not differ significantly. Within the PLD group, those with grossly enlarged livers (htTLV >3200 mL/m,  $n = 10$ ) had higher levels of GCA (1.2-fold,  $p = .02$ ), TCA (1.5-fold,  $p = .003$ ), TCDCA (1.3-fold,  $p = .003$ ) and GCDCA (1.2-fold,  $p = .003$ ) than those with smaller livers (htTLV <1600 mL/m,  $n = 63$ ).

### 3.2.2 | Comparison of changes in serum BA levels during 120 weeks of follow-up between the PLD group ( $n = 99$ ) and the non-PLD group ( $n = 48$ ), regardless of treatment

Descriptive data of all BAs are summarized in Table S1. Regardless of treatment, we evaluated changes in serum BA levels over time in PLD and non-PLD patients. In PLD patients, serum levels of the primary conjugated BAs GCDCA (1.1-fold,  $p = .002$ ) and GCA (1.1-fold,  $p = .009$ ), as well as the secondary conjugated BAs TDCA (1.2-fold,  $p = .01$ ) and GDCA (1.1-fold,  $p = .003$ ) decreased



**FIGURE 1** Differences in baseline serum bile acid levels GCA (A), TCA (B) and TCDCA (C) between polycystic liver disease (PLD;  $n=105$ ) and non-PLD ( $n=52$ ) patients. Data are log-transformed and reported as mean values with range.  $p$  Values were calculated using independent  $t$  test.

significantly over the follow-up period. UDCA on the other hand showed a non-significant increase over time. In non-PLD patients, there was a significant increase in UDCA (1.2-fold,  $p=.03$ ) and CDCA (1.2-fold,  $p=.003$ ) (Figure 2).

### 3.2.3 | Comparison of the change in serum BA levels in PLD patients who had been treated with either lanreotide ( $n=51$ ) or standard care ( $n=48$ ) and analysis of the correlation between changes in serum BA levels and changes in htTLV

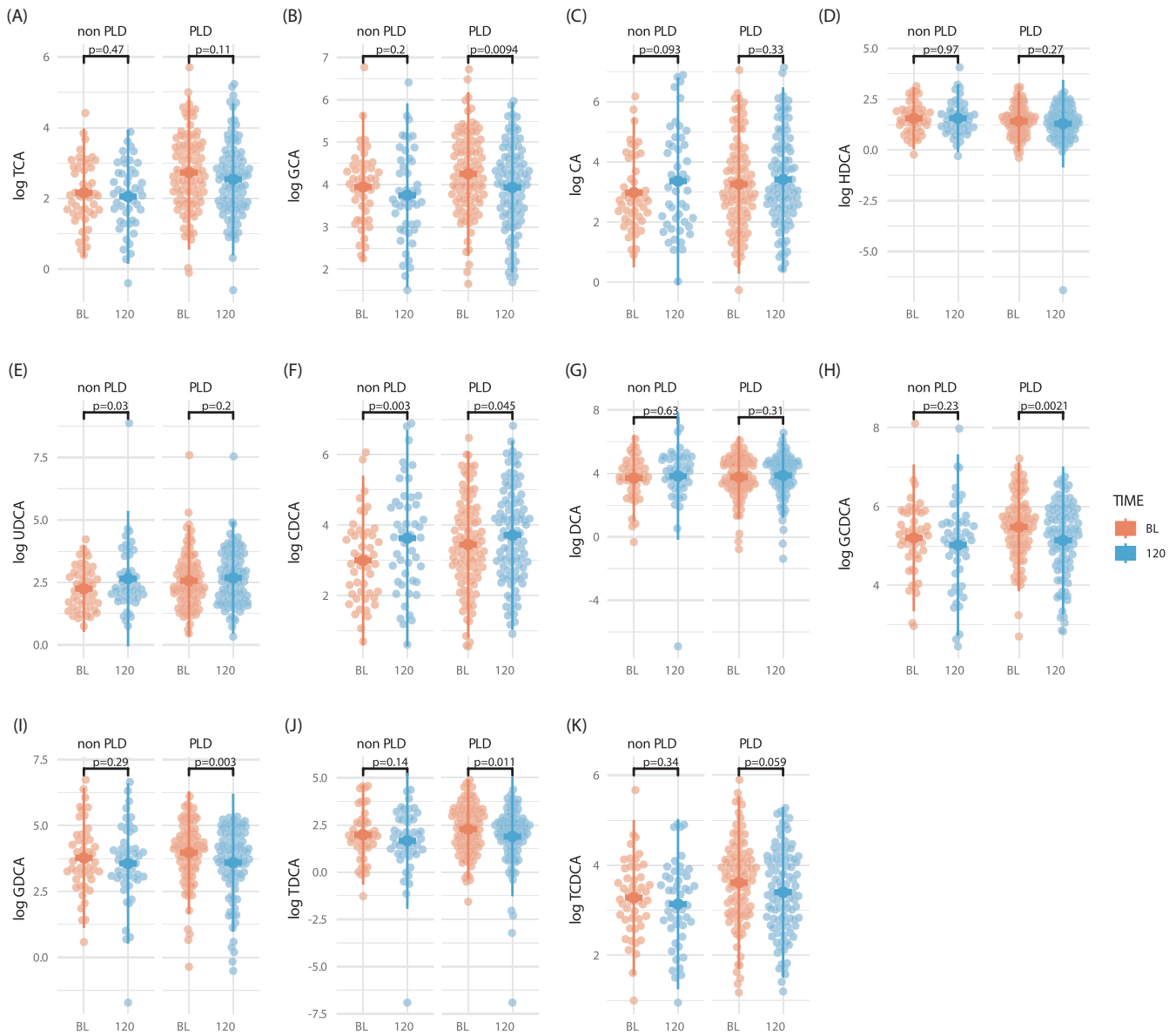
In the PLD group, pre-treatment baseline BA levels were similar between both groups. After 120 weeks of treatment with lanreotide, levels of the primary conjugated BAs TCA (1.3-fold,  $p=.002$ ), TCDCA (1.2-fold,  $p=.001$ ), GCA (1.2-fold,  $p<.001$ ) and GCDCA (1.2-fold,  $p<.001$ ), and the secondary conjugated BAs TDCA (1.4-fold,  $p=.001$ ) and GDCA (1.2-fold,  $p=.001$ ) decreased, and the unconjugated primary BA CDCA (1.1-fold,  $p=.04$ ) increased. Such changes were not seen in patients who received standard care, whose BA levels remained stable from baseline to week 120. Figure 3 depicts BA levels in PLD patients stratified by treatment group. At baseline, htTLV was similar in patients who later received lanreotide or standard care ( $p=.07$ ). In those on lanreotide, median baseline htTLV was 1594 mL/m (IQR 1258–2002) before treatment with lanreotide and 1482 mL/m (IQR 1204–2052) after treatment. In the control, group htTLV increased from 1362 mL/m (IQR 1219–1677) to 1482 mL/m (IQR 1297–1826). This resulted in an absolute change in htTLV of  $-50$  mL/m (IQR  $-129$  to  $-69$ )

versus 89 mL/m (IQR 6–179;  $p=.001$ ), and a median percentage change of  $-2.0\%$  versus  $5.8\%$  ( $p<.001$ ). We analysed whether baseline BAs associated with the change in htTLV ( $\Delta$ htTLV). No associations between baseline BAs and  $\Delta$ htTLV were found in either the lanreotide or standard care group. Finally, we analysed whether time-related changes in BAs ( $\Delta$ BA) correlated with  $\Delta$ htTLV. No associations between  $\Delta$ htTLV and  $\Delta$ BA were seen in either of the groups.

## 3.3 | Urine BAs

### 3.3.1 | Analysis of the correlation between baseline urine BAs and renal measures of outcome ( $n=145$ ) and comparison of baseline urine BAs in PLD ( $n=99$ ) and non-PLD ( $n=46$ ) patients

Descriptive data are summarized in Table S2. In an analysis of all ADPKD patients ( $n=145$ ), none of the evaluated baseline urine BAs or their fractional excretions correlated with baseline eGFR, yearly eGFR slope or baseline htTKV. In the PLD group, there was a weak association between baseline fractional CA excretion and yearly eGFR slope ( $r=-.236$ ,  $p=.03$ ) and between baseline fractional CA excretion and htTKV ( $r=.525$ ,  $p=.02$ ). However, there were no other correlations between urine BAs or their fractional excretion and renal measures of outcome. PLD patients had a significantly higher level of urine TCA/creatinine (2.2-fold,  $p<.001$ ) than non-PLD patients (Figure S3). There were no differences in the fractional excretion of BAs between PLD and non-PLD patients.



**FIGURE 2** Differences in serum bile levels of individual bile acids (A–K) between baseline (BL) and week 120 in both polycystic liver disease (PLD;  $n=99$ ) and non-PLD ( $n=48$ ) patients. Data are log-transformed and reported as mean values with range.  $p$  Values were calculated using paired-sample  $t$  test.

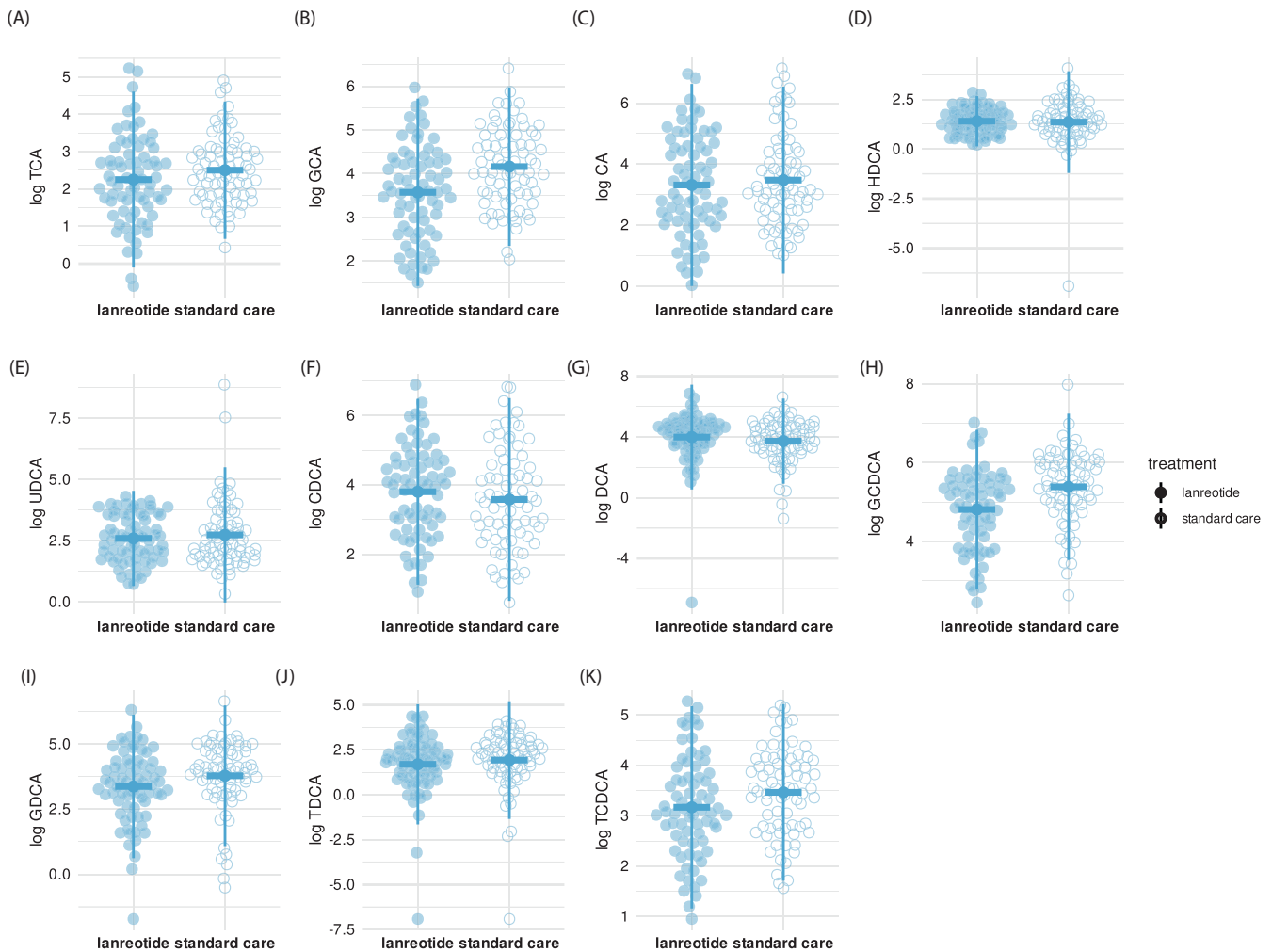
### 3.3.2 | Comparison of the change in urine BA levels during 120 weeks of follow

Urine BA levels and their fractional excretion did not change over time in the total study population ( $n=136$ ) or in the separate groups of PLD ( $n=48$ ) and non-PLD patients ( $n=23$ ). In those treated with lanreotide (PLD  $n=48$  and non-PLD  $n=23$ ), there were no associations between fractional excretion at week 120 and renal disease outcomes (baseline eGFR, baseline htTLV, yearly eGFR slope). The result for those on standard care was similar.

## 4 | DISCUSSION

We measured serum BAs in patients with PLD due to ADPKD and explored the relation between BAs, liver volume and liver growth. In addition, we studied the effect of treatment with the somatostatin analogue lanreotide on BAs, which is known to reduce liver volume. We found that baseline serum levels of taurine- and glycine-conjugated BAs were higher in patients with larger polycystic livers. Levels decreased upon treatment with lanreotide as did liver volume.<sup>5</sup> The decrease in BAs however did not correlate in a statistically significant manner





**FIGURE 3** Serum bile acid levels of individual bile acids (A–K) at week 120 in polycystic liver disease (PLD;  $n=99$ ) patients, stratified by treatment group (lanreotide,  $n=51$ ; standard care,  $n=48$ ). Data are log-transformed and reported as mean values with range.

with the change in liver volume. We also measured BAs in urine and found that urine BA levels and fractional BA excretion did not change over time and did not correlate with renal disease progression.

Our results are in line with human data on serum BAs in other types of liver disease. Under physiological conditions, the fasting total BA concentration in peripheral blood is low (range 0–10  $\mu\text{mol/L}$ ) due to a balanced regulation of intestinal absorption and hepatic and renal elimination of bile acids.<sup>16,32</sup> Postprandial levels are reported to be higher.<sup>11,33,34</sup> In patients with impaired renal function and end-stage renal disease, some BAs increase in serum while others decrease.<sup>35,36</sup> As methods for measuring BAs vary, a comparison with external cohorts needs to be interpreted with the necessary caution. In Table S3, normal values from the human metabolome database are shown<sup>37</sup> next to our results for the non-PLD ADPKD patients. Aberrant BA concentrations are associated with several types of liver disease, with or without biochemical jaundice.<sup>11,38–44</sup> Studies including patients with biliary

obstruction,<sup>44</sup> intrahepatic cholestasis of pregnancy<sup>40</sup> or alcoholic-induced hepatitis/cirrhosis<sup>41–43</sup> have reported increased serum BA levels, mainly taurine- and glycine-conjugated BAs.

Current research on the relation between serum BAs and PLD has mainly been conducted in animals.<sup>22–24</sup> Munoz-Garrido et al.<sup>22</sup> determined BA concentrations from PLD cystic fluid and compared these with the levels from paired samples of peripheral blood. In patients with PLD, BA concentrations were higher in cystic fluid than in serum. A rat model of PLD (PCK rats) has shown similar results, with increased hepatic Cyp7a1 mRNA levels, higher intrahepatic BA levels, diminished BA levels in bile and higher serum levels compared to wild-type rats.<sup>22</sup> The hepatic accumulation seemed to be caused by impaired secretion instead of enhanced hepatic synthesis. Data on the role of BAs in PLD pathogenesis are limited. A study in PCK rats has shown that increased serum BAs correlate with liver volume and impairment.<sup>23</sup> Interestingly, TGR5 (Takeda G protein-coupled

receptor 5), a G protein-coupled bile acid receptor linked to cAMP signalling, is overexpressed and mislocated in cholangiocytes of PCK rats.<sup>45</sup> This receptor is stimulated by bile acids.<sup>46</sup> In PLD, there is an overexpression of TGR5. When activated, it accelerates cAMP-dependent hepatic cyst growth.<sup>47</sup> Another recent finding is that the endogenous hydrophilic bile acid ursodeoxycholic acid (UDCA) decreases hepatic cystogenesis in PCK rats. This beneficial effect was associated with downregulation of the high concentration of cytotoxic bile acids found in PCK rat livers. Furthermore, *in vitro*, GDCA promotes the proliferation of polycystic human cholangiocytes.<sup>22</sup> In a trial in humans with PLD, UDCA did not affect liver volume, but it did reduce the growth of hepatic cysts in ADPKD patients.<sup>48</sup> Our study design is not suitable for an unequivocal causal or mechanistic/biochemical interpretation of the findings. Somatostatin and somatostatin analogues inhibit bile secretion and decrease bile flow by inhibiting bicarbonate secretion by cholangiocytes.<sup>49</sup> Intravenous administration of octreotide to patients with isolated biliary fistula results in a decrease of bile flow, but an increase in bile acid concentration in bile.<sup>50</sup> This is consistent with data that show that duodenal bile acid levels drop to 25%–33% of control values some 10–80 min after intravenous administration of octreotide to healthy subjects.<sup>51</sup> We could not measure intrahepatic or bile BA concentrations and can only speculate on the way in which lanreotide reduces peripheral blood concentrations of BAs.

It is likely that BAs are freely filtered by the glomerulus, since their molecular weight is approximately 400 Da<sup>52</sup> and the cut-off for glomerular filtration is 30–50 kDa.<sup>53</sup> After glomerular filtration, there is near complete resorption of BAs in the proximal renal tubule under normal circumstances. In contrast to our hypothesis, urine BAs and their fractional excretions remained stable over time and did not associate with renal disease progression, regardless of an intervention. Patients in this study were preselected and matched based on a relative preserved renal function (mean eGFR 57 mL/min/1.73 m<sup>2</sup>) to reduce the potential influence of decreased glomerular filtration rate on BA levels.<sup>35</sup> Whether renal function plays a role in the interpretation of our results remains uncertain, since healthy controls with preserved renal function were not included in the study design. Despite mild renal insufficiency, our study population could be identified as having a fast progressive renal disease course based on a mean annualized eGFR slope in PLD and non-PLD patients of  $-3.2$  and  $-3.7$  mL/min/1.73 m<sup>2</sup>/year, respectively. The lack of an association between urine BAs and renal disease progression might be explained by the absence of aberrant urinary excretion of toxic BAs in our study population. Therefore, we cannot draw a firm

conclusion regarding the urine BAs and renal disease progression.

The strength of our study is that we included a phenotypically well-defined cohort of patients with ADPKD and PLD who participated in a multicentre, randomized clinical trial,<sup>27</sup> with prospectively defined outcomes including data on total liver volume and follow-up data of 2.5 years. In de DIPAK-1 trial, the effect of lanreotide was investigated, and therefore, treatment allocation was included in our analyses. Furthermore, we determined the levels of separate BAs, both in serum and urine samples at different time-points. Samples were collected and stored using standardized procedures, limiting potential bias.

In conclusion, in ADPKD patients with PLD, baseline serum BAs were positively associated with htTLV, but not with liver growth. Lanreotide reduced BA levels and has previously been shown to reduce htTLV. However, in this study, the decrease in BA levels was not associated with the change in liver volume. Future (interventional) studies that look to influence receptors for BAs in cholangiocytes or bile composition or the flow of bile, would do well to measure BAs, in the liver and blood in animal models and in the blood in humans. This will help to understand the role of BAs in the pathophysiology of PLD.

#### AUTHOR CONTRIBUTIONS

S.D.: Conceptualization, Methodology, Validation, Formal analysis, Data curation and Writing (original draft). J.B.: Investigation and Writing (review and editing). M.G.: Investigation and Writing (review and editing). N.B.: Investigation and Writing (review and editing). J.D.: Writing (review and editing). O.M.: Conceptualization, Methodology, Formal analysis, Writing (review and editing) and Visualization. J.F.: Conceptualization, Methodology, Validation, Resources, Writing (review and editing) and Supervision. D.S.: Conceptualization, Methodology, Validation, Writing (review and editing) and Supervision.

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## CONFLICT OF INTEREST STATEMENT

None.

## DATA AVAILABILITY STATEMENT

The patient data used for this study are restricted and not publicly available due to privacy and ethical concerns. The data underlying this article may potentially be shared on reasonable request to the corresponding author and with appropriate permissions and oversight.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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